LIF is a basic and heavily glycosylated monomeric protein. LIF is known by a variety of alternative names and exerts diverse effects upon haemopoietic cells, embryonal stem cells, primitive germ cells, hepatocytes, neurones, adipocytes, myoblasts and osteoblasts. Consistent with its broad biological effects in vitro, elevation of LIF levels in vivo results in a complex pathology including wasting, elevated megakaryocyte and platelet numbers, and aberrant bone deposition. Although LIF is pleiotropic, mice that fail to produce this cytokine as a consequence of targeted disruption of the gene appear to mature normally. Females that fail to produce LIF, although capable of becoming pregnant, do not carry litters to term. The defect in this case is independent of the genotype of the embryo and resides in the inability of embryos to implant correctly in the uterine wall.

Alternative names

Differentiation inducing factor (DIF), differentiation stimulating factor (D-factor), differentiation inhibitory activity (DIA), differentiation retarding factor (DRF), cholinergic neuronal differentiating factor (CNDF), human interleukin for DA cells (HILDA), hepatocyte stimulating factor-III (HSF-III), melanocyte derived lipoprotein lipase inhibitor (MLPLI). (Hilton 1992.)

LIF protein

LIF is a secreted protein that is heavily glycosylated and quite basic (p*I* > 9.0) (Hilton 1992). As a native protein produced by mammalian cells, LIF exhibits an apparent molecular weight of 32 000 to 62 000 Da, depending on the source, but deglycosylation reduces this to 20–25 000 Da, similar to the molecular weight predicted from the cDNA sequence (Hilton 1992). Recombinant LIF produced in yeast is hyperglycosylated while that produced in *E. coli* is not glycosylated (Hilton 1992). The glycosylation of LIF does not appear to affect its biological activity either *in vitro* or *in vivo*. LIF contains six cysteine residues each of which are conserved across species. The pattern of disulphide-bond formation has been determined for murine LIF (Nicola *et al.* 1993): C13–C135, C19–C132, and C61–C164. A similar arrangement is observed for the two disulphide bonds of human oncostatin-M (Rose and Bruce 1991). Although LIF exhibits only a weak primary sequence similarity to oncostatin-M and other cytokines, these proteins are thought to form a family composed of four alpha-helical bundle structures (Bazan 1991).

Native LIF has been purified from a number of sources using conventional chromatography (Hilton 1992). The various purification schemes take advantage of LIF's high isoelectric point and use anion exchange chromatography as an early step (Hilton 1992). More recently recombinant LIF has been produced as a fusion product with glutathione-S transferase and can be readily purified using immobilized glutathione (Gearing *et al.* 1989).

LIF sequence and gene

cDNA molecules encoding human, murine, rat, porcine and ovine LIF have been cloned and sequenced (Hilton 1992). The primary mRNA species is 4.0–5.0 kb in length (Hilton 1992). LIFs from different species exhibit between 78 and 92 per cent amino acid sequence identity (Fig. 1), but while human LIF seems able to act on both human and murine cells, murine LIF is not active on human cells (Layton *et al.* 1992).

The genes encoding human and murine LIF are located on chromosomes 22q12 and 11A1, respectively (Hilton 1992). They contain three exons and two introns, are similar in structure, and are approximately 6 kbp in length. mRNA species utilizing alternative first exons have been described (Rathjen *et al.* 1990). These mRNA species give rise to proteins that differ in the first few amino acids of the signal peptide. This difference has been claimed to influence the extracellular location of secreted LIF (Rathjen *et al.* 1990).

The GenBank database accession numbers for human and mouse LIF cDNA and genomic sequences are X13987, X06381, M63419, S05435, M63420, and S05436.

LIF production

LIF is normally undetectable in both mouse and human serum (Hilton 1992). LIF is, however, detectable upon injection of mice with lipopolysaccharide and is found in the serum of patients with florid infections (Hilton 1992; Metcalf 1992). In these situations LIF appears to be produced by a wide range of tissues (Gough *et al.* 1992). Of particular interest in normal mice is the spike of production observed in the endometrial glands of the uterus at the time of embryo implantation (Bhatt *et al.* 1991).

Consistent with the multiple primary sources of LIF, many cell lines are capable of producing LIF (Hilton 1992). These include the human bladder carcinoma cell line 5637, human melanoma line SEKI, and the colon carcinoma cell line COLO-16. The following rodent cell lines also produce LIF:

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              10        20         30 #       40        50       # 60
M  MKVLAAGIVPLLLLVLHWKHGAGSPLPITPVNATCAIRHPCHGNLMNQIKNQLAQLNGSA
R  MKVLAAGIVP-LLLILHWKHGAGSPLPITPVNATCAIRHPCHGNLMNQIKSQLAQLNGSA
H  MKVLAAGVVP-LLLVLHWKHGAGSPLPITPVNATCAIRHPCHNNLMNQIRSQLAQLNGSA
O  MKILAAGVVP-LLLVLHWKPGAGSPLPINPVNATCNTHHPCPSNLMSQIRSQLAQLNGTA
P  MKVLAAGVVP-LLLVLHWKHGAGSPLSITPVNATCATRHPCHSNLMNQIKNQLAHVNSSA

          70        80    #  90     # 100       110         #
M  NALFISYYTAQGEPFPNNVEKLCAPNMTDFPSFHGNGTEKTKLVELYRMVAYLSASLTNI
R  NALFISYYTAQGEPFPNNVDKLCAPNMTDFPPFHANGTEKTKLVELYRMVTYLGASLTNI
H  NALFILYYTAQGEPFPNNLDKLCGPNVTDFPPFHANGTEKAKLVELYRIVVYLGTSLGNI
O  NALFILYYTAQGEPFPNNLDKLCGPNVTDFPPFQPNGTEKVRLVELYRIVAYLGTALGNI
P  NALFILYYTAQGEPFPNNLDKLCGPNVTNFPPFHANGTEKARLVELYRIIAYLGASLGNI

          #          #          150       160       170       180
M  TRDQKVLNPTAVSLQVKLNATIDVMRGLLSNVLCRLCNKYRVGHVDVPPVPDHSDKEAFQ
R  TWDQKNLNPTAVSLQIKLNATTDVMRGLLSSVLCRLCNKYHVGHVDVPCVPDNSSKEAFQ
H  TRDQKILNPSALSLHSKLNATADILRGLLSNVLCRLCSKYHVGHVDVTYGPDTSGKDVFQ
O  TRDQKTLNPTAHSLHSKLNATADTLRGLLSNVLCRLCSKYHVAHVDVAYGPDTSGKDVFQ
P  TRDQRSLNPGAVNLHSKLNATADSMRGLLSNVLCRLCNKYHVAHVDVAYGPDTSGKDVFQ

          190       200
M  RKKLGCQLLGTYKQVISVVVQAF
R  RKKLGCQLLGTYKQVISVVVQAF
H  KKKLGCQLLGKYKQIIAVLAQAF
O  KKKLGCQLLGKYKQVMAVLAQAF
P  KKKLGCQLLGKYKQVISVLARAF
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Figure 1. Alignment of the predicted amino acid sequence of human, murine, rat, porcine, and ovine LIF. The predicted signal sequence is shown by a solid line above the sequence. Conserved cysteine residues are shown in bold type. Potential N-linked glycosylation sites are indicated by #.

Krebs and Ehrlich's ascites cells, L929, T-lymphoid lines (eg. EL-4 (?)B-3), and STO fibroblasts (Hilton 1992; Gough et al. 1992).

In vitro biological effects of LIF

The biological effects described for LIF *in vitro* are bewildering in their number and diversity (Hilton 1992; Metcalf 1992). LIF has been shown to induce the differentiation and suppress the clonogenicity of the mouse monocytic leukaemia cell-line M1. LIF also synergistically suppresses the clonogenicity of the human leukaemic cell-lines HL-60 and U937. The induction of M1 differentiation is the assay by which LIF activity is usually quantitated. The concentration of LIF that induces 50 per cent of M1 colonies to differentiate in a semi-solid agar culture is defined as 50 U/ml. Purified native and recombinant LIF have a specific activity of 1 to 2 × 10^8 U/mg.

A most widespread use of LIF is in the suppression of embryonic stem cell differentiation (Hilton 1992; Metcalf 1992). In the presence of LIF these cells may be manipulated genetically, for example by targeted disruption of genes by homologous recombination, and subsequently re-introduced into the blastocysts of recipient embryos where they contribute to all tissues, including the germline. LIF enhances the survival and proliferation of primitive germ cells (Matsui et al. 1991). LIF stimulates acute phase protein synthesis by HepG2 cells but inhibits lipoprotein lipase activity in 3T3-L1 cells (Hilton 1992; Metcalf 1992). It induces a cholinergic phenotype in adrenergic neurones and enhances the survival and proliferation of embryonic sensory neurones and myoblasts (Hilton 1992; Metcalf 1992). LIF stimulates the proliferation of the factor-dependent haemopoietic cell lines DA-1a and THP-1, enhances the generation of megakaryocytes by interleukin-3 and has been suggested to increase the frequency of infection of haemopoietic stem cells with retrovirus—perhaps by stimulating these cells to enter the cell cycle (Hilton 1992; Metcalf 1992).

In vivo actions of LIF

The pleiotropy and redundancy of LIF's biological effects are well-illustrated by comparing mice in which LIF levels

have been artificially elevated (Metcalf 1992) to those that are unable to produce LIF because of disruption of the LIF gene by homologous recombination (Stewart *et al.* 1992).

LIF levels have been elevated in mice by injection of purified recombinant LIF and by engrafting mice with LIF-producing haemopoietic cell (Metcalf 1992). The effects in both cases are similar. There is an almost complete loss of subcutaneous and abdominal fat—resulting in a 30 per cent reduction in weight within three days (Metcalf 1992). Mice have elevated serum calcium levels, and in some cases excessive deposition of new bone and calcification of skeletal muscle, heart, and liver (Metcalf 1992). Symptoms of an ongoing acute-phase response are observed with a decrease in serum albumin concentration and an increase in erythrocyte sedimentation rate (Metcalf 1992). Platelet levels are increased in mice, and also primates, with increased circulating LIF levels, as are the numbers of megakaryocytes and megakaryocyte progenitors in the spleen (Metcalf 1992).

In contrast to the elevation of LIF levels, there appear to be very few detrimental effects of the failure to produce LIF (Stewart *et al.* 1992). Mice lacking a functional LIF gene appear to develop normally and have a normal lifespan. Mice also appear to be histologically normal. The most profound effect, however, is on the implantation of embryos in the uteri. Embryos, irrespective of their capacity to produce LIF, are unable to implant in the uteri of female mice that do not produce this cytokine (Stewart *et al.* 1992). This defect can be corrected by injection of purified LIF into the uterus at the time of implantation (Stewart *et al.* 1992).

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OSM is a
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